TOTAL LYMPHOCYTE COUNT AS AN ALTERNATIVE TO CD4 COUNT IN MANAGMENT OF HIV/AIDS PATIENTS

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ABSTRACT

Background: Initiation and monitoring of ART are based on CD4+ count which is however, costly and often inaccessible in resource restricted communities. TLC (total lymphocyte count) has been advocated over the years as a marker for progression of HIV. The aim of the study was to find relationship between CD4 count and TLC and to determine whether TLC can be used as a surrogate marker for CD4 counts.

Methods: Descriptive/Cross-sectional study was conducted at department of Pathology Allama Iqbal Medical College Lahore. A total of 106 HIV subjects were included. Blood samples were analyzed for TLC and CD4 counts. Pearson's correlation between TLC and CD4 count was evaluated. Receiving Operating Characteristic (ROC) was used to calculate sensitivity, specificity, positive and negative predictive values for various cut-off points of TLC to predict CD4 count ≥500/µl, 200–499/µl, <350/µl and < 200/µl.

Results: A TLC of ≤1400/µl had a maximum sensitivity of 83.3% and specificity 71.1% for predicting CD4 cell count of < 200/µl. The best TLC cut-off for predicting CD4 count <350/µl with a maximum sensitivity of 81.5% and specificity 76.4% was ≤2200 /µl. A CD4 count ≥500/µl was predicted with maximal sensitivity of 88% and specificity of 73.2% at TLC cut-off >2200 /µl. A positive Pearson's correlation coefficient (r) of 0.6623 ($p < 0.0001$) was noted when TLC and CD4 count were analyzed. Area Under Curve of different groups was high (close to 1) that makes TLC an ideal alternate to CD4 count.

Conclusion: We suggest cut-off TLC ≤1400/µl for anticipating CD4 counts < 200/µl to initiate ART in resource-poor settings.

Key Words: CD4 count, Total lymphocyte count, HIV

How to cite this article: Javaid MI, Ghazanfar M, Rehman Fatima. Total Lymphocyte Count as an Alternative to Cd4 Count in Management of HIV/Aids Patients. Pak Postgrad Med J 2024;35(2): 73-76

DOI: https://doi.org/10.51642/ppmj.v35i02.602

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INTRODUCTION

Individuals living with Human Immunodeficiency Virus were estimated around 37.9 million worldwide in 2018.[1] Most individuals living with HIV infection are from developing nations.[2] In Pakistan, it is estimated that HIV prevalence among the general population is less than 0.1%, with 165 000 individuals residing with HIV[3] and less than 5% individuals with HIV are receiving ART (antiretroviral therapy) in developing countries.[2]

CD4 count is a test of the immune status of HIV-infected people that more appropriately measures the intensity of immunosuppression than the clinical stage of disease. Therefore, this threshold marker for the initiation of ART is included in the international guidelines.[4] Current recommendations for commencement and monitoring of ART in western countries are based on CD4+ T-cell counts and HIV viral burden. These techniques, however, are costly[5] and in addition, a precise measurement of the CD4 cell count requires flow cytometry that is often inaccessible in resource-limited communities for a wide variety of reasons.[4] Therefore, World Health Organization (WHO) mandates that CD4 count measurement is "desirable" but not necessary to initiate ART in resource-limited countries.[5] In the absence of CD4 monitoring, WHO guidelines have formerly suggested clinical staging either alone or combined with an absolute lymphocyte count to determine the eligibility for ART.[4]

According to the WHO and Center for Disease Control (CDC), the commencement of antiretroviral therapy was based on CD4 counts <350 cells/µl.[2] In recent times WHO guidelines use a high CD4 count (≤500 cells/µl) than earlier (≤350 cells/µl to determine ART eligibility.[4, 6] In areas where resources are limited, it has been proposed that, in addition to assessing the clinical stage of HIV, a total lymphocyte count (TLC) of less than 1200 cells per microliter should be used as a threshold for beginning antiretroviral therapy[2].

The utility of TLC has been advocated over the years as an indicator of HIV progression.[7, 8] Studies have proposed that when used in combination with hemoglobin, the total lymphocyte count becomes a more sensitive marker for progression of HIV while other researches invalidate the use of TLC in such settings.[9-12]

The discrepancies observed in various study contexts necessitated the undertaking of this research. The aim of this study was to investigate the correlation between CD4 count and TLC and to determine if TLC could be a substitute marker for CD4 count in low-resource areas in Pakistan.

METHODS

This research, which used a descriptive and cross-sectional approach, took place at the Department of Pathology in Allama Iqbal Medical College located in

Lahore. The collection of blood samples from participants occurred before the initiation of ART (antiretroviral therapy). The Ethical Review board of Allama Iqbal Medical College approved this study. In accordance with the Helsinki Declaration, informed consent was obtained on written forms all participants of the study. Demographic questionnaires were completed once consent was obtained. A Vacutainer tube containing Ethylenediaminetetraacetic acid (EDTA) was used to collect a 5 ml sample of blood, which was then analyzed for CD4 T-cell count and total leukocyte count. Within 2 to 4 hours of collection, patient samples were analyzed. The TLC was measured through an automated blood analyzer called Sysmex Kx-21, while the CD4+ T lymphocytes count was determined using the Becton Dickinson (BD) FACS Calibur. The BD FACS Calibur used flow cytometry for the quantification of the CD4+ T Lymphocytes by a monoclonal antibody cocktail comprised of CD3 PerCp, CD4 FITC and CD8 PE in a TruCount tube.

After providing informed consent, a total of 106 HIV seropositive participants referred from the Punjab AIDS Control Programme (PACP) were enrolled in the study. The study participants were divided into three groups based on the Centres for Disease Control and Prevention Criteria (CDC), which emphasises the relevance of CD4 + T cell testing in the clinical management of HIV-infected patients. The groups were as follows: (1) CD4 counts 500 cells/µl; (2) 200-499 cells/µl; and (3) 200 cells/µl and TLC cut-off values were established for the said groups. We also analyzed threshold cut- off TLC value for CD4 count group <350 cells/µl. The age requirement for inclusion was at least 18 years old with HIV seropositive with all genders referred from Punjab AIDS control Programme. Antiretroviral therapy use, self-reporting, and co-morbidity with other illnesses (such as tuberculosis, endocarditis, congenital immune disorders, and acute viral infections) that could significantly alter hematologic parameters were all grounds for exclusion.

Results were presented as mean ± SD. The Pearson's correlation test was used to assess correlations. For several cut-off points of the TLC to predict CD4+ T-cell count 500 cells/µl, 200-499 cells/µl, 350 cells/µl, and 200 cells/µl, Receiver Operating Characteristic (ROC) was used to determine Sensitivity, specificity, positive and negative predictive values with 95% confidence intervals (CIs). $P < 0.05$ was chosen as the significance level for all statistical amylases. MedCalc and SPSS-23 were used to analyze data.

RESULTS

Table 1 displays the subjects' demographic information. This demonstrates mean age, CD4+ count and Total Lymphocyte Count (TLC) in 3 groups, expressed as means ±1SD.

Table 1 The demographic features of study population with HIV infection.

	CD4+ COUNT (cells/µl)		
	< 200	200-499	≥ 500
Age (Years)	34.7±8.6	31.5±9.1	30.1±8
Number of Subjects	18(17%)	38(35.8%)	50(47.2%)
Gender			
Male	15(18.1%)	25(30.1%)	43(51.8%)
Female	3(17.6%)	10(58.8%)	4(23.5%)
Trans-Gender	0	3(50%)	3(50%)
Lymphocyte x 10^3(cells/µl)	1100±600	2300±800	3100±800
CD^{4+} (cells/µl)	81.9±52.7	332.2±91.8	737.1±203.1

Mean ±1SD is used to express the values.

Tables 2 illustrate the sensitivity, specificity, positive and negative predictive values of various TLC cut-offs for CD4 groups. Based on the optimal TLC cut-off values, which have the highest sensitivity and specificity in combinations, a TLC of ≤ 1400 cells/µl was shown to have 83.3% sensitivity and 71.1% specificity for predicting a CD4 cell count of <200 cells/µl. The optimum TLC cut-off for predicting CD4 count <350 cells/µl was ≤2200 cells/µl, with a maximum sensitivity of 81.5% and specificity of 76.4%. At a TLC cut off of >2200 cells/µl, a CD4 count of ≥500 cells/µl was predicted with a maximum sensitivity of 88% and specificity of 73.2% and CD4 count between 200-499 cells/µl with sensitivity of 65.7% and specificity of 64.7% at TLC ≤2300 cells/µl as shown in Table 2.

Table 2 Sensitivity, Specificity, Positive and Negative predictive values for TLC cut-offs as per CDC classification for different $CD4^+$ count categories.

	TLC Cut- off *(cells/µl)*	Sensitivity (%)	Specificity (%)	PPV %	NPV %
CD^{4+} count <200 *(cells/µl)*	≤1100	66.67	98.86	92.3	93.5
	≤1200	66.67	97.73	85.7	93.5
	≤1300	77.78	96.59	82.4	95.5
	≤1400	83.33	93.18	71.4	96.5
	≤1500	83.33	90.91	65.2	96.4
CD^{4+}count 200-499 *(cells/µl)*	≤2100	52.63	67.65	47.6	71.9
	≤2200	63.16	66.18	51.1	76.3
	≤2300	65.79	64.71	51.0	77.2
	≤2400	65.79	60.29	48.1	75.9
	≤2500	68.42	52.94	44.8	75.0
CD^{4+} count ≥500 *(cells/µl)*	>2100	90.00	66.07	70.3	88.1
	>2200	88.00	73.21	74.6	87.2
	>2300	86.00	75.00	75.4	85.7
	>2400	80.00	75.00	74.1	80.8
	>2500	70.00	76.79	72.9	74.1
CD^{4+} count <350 *(cells/µl)*	≤1900	65.79	85.29	71.4	81.7
	≤2000	73.68	80.88	68.3	84.6
	≤2100	73.68	79.41	66.7	84.4
	≤2200	81.58	76.47	66.0	88.1
	≤2400	81.58	69.12	59.6	87.0

Total lymphocyte count-TLC; Predictive positive value-PPV; Negative predictive value-NPV

Figure 1 depicts the ROC curves for various CD4 count groups. Figure 1 also depicts the areas under the curves (AUC) for the various CD4 groupings. The Area Under Curve of the different groups was close to 1, indicating that TLC is an excellent alternative for CD4 count.

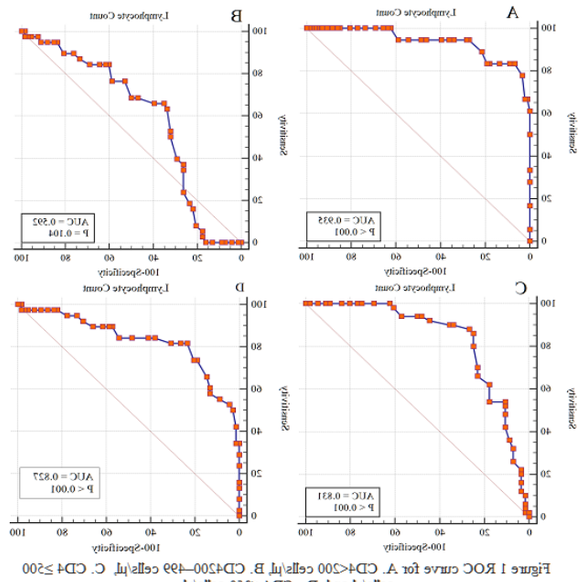

Figure 1 ROC curve for A. CD4<200 cells/µl, B. CD4200—499 cells/µl, C. CD4 ≥500 cells/µl and D. CD4 ≥350 cells/µl

TLC and CD4 count analysis revealed a positive Pearson's correlation coefficient (r) of 0.6623 (p <0.0001) as shown in Figure 2.

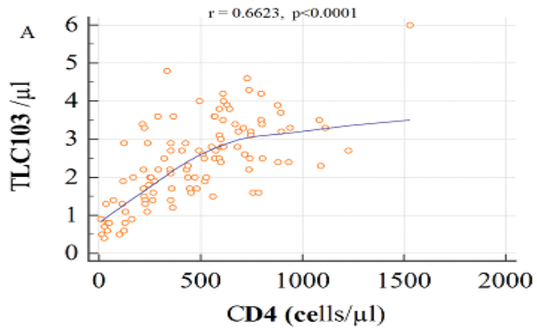

Figure 2 Correlation between Total Lymphocyte Count and CD4 Count

DISCUSSION

Depletion of total lymphocytes (mainly due to its subset CD4 cells) was recognised as a sign of HIV infection.[13] The gold standard for determining the stage of HIV/AIDS is the CD4 count, guiding HIV-infected people's treatment choices and evaluating efficacy of therapy. It has been argued that a cut-off value for TLC should be utilised as a substitute marker for CD4 in staging, monitoring, and therapeutic options in resource-limited settings.[12]

Many researchers worldwide are determined on assessing the utility of TLC as the surrogate marker of a CD4 count below 200 cells/µl for HIV-infected subjects of diverse race and ethnicity.[14]

In this study, the threshold analysis was carried out to determine TLC's ability to forecast CD4 counts at various levels, i.e. CD4 cells <200/µl, CD4 cells 200–499/µl, CD4 cells< 350/µl and CD4 cells < 500/µl.

TLC of ≤1200/µl, as recommended by WHO, exhibited a sensitivity of 66.67%, a specificity of 97.73%, a positive predictive value of 71.4%, and a negative predictive value of 96.5% in our study. According to our knowledge several researches had shown less sensitivity of TLC ≤1200 cells/µl to predict CD4 < 200 cells/µl.[5, 8, 12, 15, 16] Although studies from Obirikorang C et al., and Karanth SS et al., demonstrated greater sensitivity (72.22% and 73%) and specificity (100% and 100%), for TLC cut-off of ≤1200/µl to infer CD4 count< 200/µl.[2, 17] This difference could have been due to different factors of ethnicity, race, epidemiology and socioeconomics.

Table 3 Comparison of the sensitivity and specificity of TLC cut-off < 1200/µl to predict CD4 count < 200 in the current research and previous researches.

Researches	Sensitivity	Specificity
Daka et al., [12]	41%	83.5%
Angelo ALD et al.,[15]	46.5%	92.8%
Karanth SS et al., [17]	73%	100%
Kakar A et al., [16]	64.4%	91.1%
Sreenivasan S et al., [5]	63.41%	69.57%
Obirikorang C et al.,[2]	72.22%	100%
Gitura et al.,[18]	33%	99%
Agrawal et al.,[14]	34.48%	67.5%
Dhamangaonkar, et al.,[19]	23.27%	86.90%
Mwamburi et al.,[20]	61%	90%
Present study	66.67%	97.73%

According to our findings, a TLC of ≤ 1400 cells/µl (higher than WHO proposed) was having maximal sensitivity 83.3% and specificity of 71.1% to expect CD4 count of <200 /µl, so at this cut off only 2 patients out of 10 will be missed with CD4 <200 cells/µl. Kumarasamy N et al. discovered that the TLC cut-off < 1400/µl had a sensitivity of 73%, 88% specificity, 76 % PPV and 86% NPV to predict < 200 cells/µl CD4 count.[8] Other studies in India also reported larger TLC cut-offs to predict < 200 cells/µl CD4 cell count.[5, 17] Researches from Brazil and Ethiopia also agreed with greater TLC cut-offs for CD4 count < 200/µl.[12, 15]

The current study found that TLC <2200/cells/µl showed a highest sensitivity of 81.58%, specificity of 76.47%, positive predictive value of 66% and negative predictive value of 88.1% to infer CD4 count <350/µl. In a comparable research, TLC < 2100/µl was reported to have the most suitable predictive power having sensitivity of 82.1%, specificity of 57.8%, PPV 79.3% and NPV 62.2%.[21]

We found significant correlation with r value 0.6623 among TLC and CD4 count ($p < 0.0001$) (Figure 2). Similar levels of correlation between CD4 count and TLC were found by Kumarasamy N et al. and other Indian studies by Karanth SS et al., Kakar A et al., and Sreenivasan S et al., with r-values of 0.744, 0.682, 0.714, and 0.560, respectively.[5, 8, 16, 17] Similarly, Fasakin et al. examined r-value 0.65 in research from other parts of the world; Daka et al. discovered correlation with r-value 0.398; and Angelo ALD et al. demonstrated r-value 0.58.[12, 15, 22] In current study, TLC attained a comparatively higher diagnostic efficiency (Area Under Curve=0.935) to predict a CD4 count < 200/µl with 83.33 percent sensitivity and 93.18 percent specificity at a threshold level of ≤ 1400 cells/µl (Table 2). Area under curve (AUC) for the calculation of the CD4 count < 350/µl was 0.827 with a sensitivity and specificity of 81.58 % and 76.47% respectively at the cut-off TLC as of ≤2200/ul. Comparable research by Chen J et al. reveals high diagnostic accuracy (Area Under Curve=0.80) to predict CD4 count < 350 /µl.[23]

CONCLUSION

We come to the conclusion that TLC is a suitable and appropriate substitute for anticipating CD4 counts < 200/µl to initiate ART in resource-poor settings. However, unlike the WHO, we suggest cut-off TLC ≤ 1400 cells/µl instead of < 1200 cells/µl. More persons requiring antiretroviral therapy were identified with TLC ≤1400 cells/µl. However, more research in resource-constrained situations with bigger research teams is needed to assess the usefulness of TLC as a substitute for CD4 counts.

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AUTHOR'S CONTRIBUTIONS

MIJ: Concept, manuscript writing, reference research
MG, FR: Data collection, data analysis, editing and approval of final draft.